

In Vitro* antitumor study on propolis and pollen as honeybee products*Rehab E. Ali^{1*}, Amany Salem¹, Aly Mahrous², Eman Mohamed³, Hassan Mohamed²**

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*Corresponding author E-mail: Rehab111111@yahoo.com**Received: November 4, 2023; Accepted: November 21, 2023; Available online: November 21, 2023****Doi: 10.21608/AJBS.2023.326814****ABSTRACT**

The present *in vitro* study aimed to investigate the ability of propolis and pollen as honey bee products in treatment of tumor. As well as, using them and their water extract as in-functional food which have pharmacological properties and antioxidant compounds. This study was performed on tumor cells (caco2 cell and hepG2 cell) *in vitro*. The chemical composition, Total phenols, Total flavonoids and antioxidant activity of DPPH were estimated. Fractionation and identification of phenolic compounds and flavonoids compounds were determined by HPLC. Also, the water extracts of propolis and pollen were used in crackers product. The results indicated that the raw propolis and pollen were higher than their water extracts for total phenols and total flavonoids. While, water extract of propolis has higher antioxidant activity by DPPH than raw samples. Moreover, raw propolis and pollen have more phenolic, flavonoid compounds contents than their water extract by HPLC. Also, raw pollen has the highest level of caffeine, oleuropin, ferulic and ellagic (36.51, 16.07, 28.41, and 18.65 mg/100g, respectively) as a phenolic compounds. Antitumor assay indicated that water extract of propolis has (IC₅₀ equal to 123.55) more than water extract of pollen (IC₅₀ is 113.61) for colon cancer (caco₂). While, water extract of pollen has (IC₅₀ equal to 825.25) more than water extract of propolis (IC₅₀ is 352.2) for liver cancer (HepG₂). The crackers samples which contained a high percent (1, 5%) of water extract of propolis or pollen has the highest scores of taste, flavor, texture and general appearance. In conclusion the results of the current study indicated that bee propolis can be used as anticancer for colon cancer and bee pollen as anticancer for liver cancer. As well as, propolis and pollen can be used in functional food.

Keywords: Propolis, pollen extract, honeybee products, antioxidant, cytotoxic effect.**INTRODUCTION**

Propolis is a natural resinous material collected by bees from different sources of plant exudates, buds, leaves, and barks. It is mainly composed of resin (50%), wax (30%), essential oils (10%), pollen (5%), and other organic compounds (5%) were used to seal holes in beehive, protect larvae, young bees, and the queen bee from microbial infection, as well as to maintain constant humidity and internal

hive temperature of around 35°C. Propolis, which is barely soluble in water, cannot be used as a raw material and it must be purified by extraction with solvents to remove the inert material and preserve the polyphenolic fraction. These last compounds, flavonoids and phenolic acids, are considered to contribute more to the healing effects than the other propolis constituents (Huang *et al.*, 2014). Propolis has pharmacological properties such as

antioxidant, anti-microbial, anti-septic, anti-inflammatory, anesthetic, anti-tumor and diabetic activities (Anjuma *et al.*, 2019).

Bee pollen has been promoted as a valuable apitherapeutic product due to its potential therapeutic value. It has greater antimutagenic properties in certain types of cancer (Munsted and Bogdanov, 2009). The anticarcinogenic activities may be derived from its antioxidant properties, i.e. suppression of oxygen reactive species (ROS) formation and removal or inactivation of oxygen reactive species (Szcze, 2006). Bee pollen ability to induce apoptosis and stimulate secretion of tumor necrosis factor-alpha (TNF- α). Thus, bee pollen may be considered to have cytotoxic activity on cells by inhibiting their development (Pascoal *et al.*, 2014).

The snacks market is an ever-expanding area, including foods such as crisps, crackers, cookies, biscuits and bars. Now more than ever, consumers are seeking broader and more nutritive functions from their snacks as they become a bigger part of their daily diet (Kim, 2017). Crackers are biscuits having typical flaky inner layers. Crackers contain little sugar, moderate levels of fat and relatively low levels of salt (Han *et al.*, 2010). Consequently, crackers can be used as a good substitute for sweeter snacks. Along with, crackers can be utilized as a source of incorporation of different nutritionally rich ingredients for the diversification (Sudha *et al.*, 2007). Among these added ingredients, dietary fiber and antioxidant has gained tremendous attention. Valencia *et al.* (2006) reported that there is an increasing demand for high fiber and antioxidant food products in order to overcome health problems such as hypertension, diabetes, and colon cancer.

The current study aims to utilize propolis and pollen as a honey Bee product in functional food and produce healthy crackers samples contained propolis, pollen extract with different levels. These

crackers were checked as a anti colon, liver and cancer or protect from tumor.

MATERIALS AND METHODS

Materials:

Crude propolis and pollen were obtained from the Local market (kingdom of Bees) Cairo. Wheat flour 72%, salt, corn oil, baking powder, milk powder were purchased from the local market in Cairo.

Chemicals:

Folin Ciocalten phenol reagent (2N), Sodium Carbonate (99.8%) (Na₂CO₃), sodium nitrite (NaNO₂), Alamonium chloride (AlCl₃), sodium hydroxide (NaOH) and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (St. Louis, Mo, USA).

Gallic acid, (4) -Catechin, α -phosphoric acid (85%), m-phosphoric acid, glacial acetic acid (35.7%), hydrochloric acid (35%) standards (phenols, flavonoids) and the HPLC solvents were purchased from fisher scientific (Fair Lawn, NJ, USA).

Extraction of propolis:

Propolis (50 g) added to (500 ml) distilled water (1:10W/V). Then filtered through Whatman No. 1 filter paper (de Lima *et al.*, 2016).

Extraction of Bee pollen:

Bee pollen milled for 5 min using a (Breville Coffee Grinder Model BCG200). An amount (50g) of pollen sample were separately extracted using 500 ml distilled water (1:10 W/V) then put in Ultrasound-assisted cleaner bath (Daihan Scientific Co. Ltd, Wonju-si, Gangwon-do, South Korea) for 15 min at 70 °C according to de Lima *et al.* (2016). The frequency of the bath was 40 kHz. Then mixture was centrifuged at 6000 g for 15 min using a centrifuge (Hettich universal 320R, Germany). The supernatant was collected in jars, -covered and storage till analysis according to Ivan *et al.* (2021).

Chemical composition for product:

Chemical composition for product (protein, fat, fiber and ash).carbohydrates

In Vitro antitumor study on propolis and pollen as honeybee products

was collected by difference as given by A.O.A.C. (2007). Determination of the total phenol and total flavonoid contents were estimated based on procedures described by Batista *et al.* (2011). Antioxidant activity by DPPH was determined by Hanato *et al.* (1988).

Fractionation of phenolic, flavonoid compounds by HPLC:

Fractionation and identification of phenolic compounds were determined by HPLC as according to the method of Goupy *et al.* (1999). Fractionation of flavonoid compounds were determined by HPLC as according to the method of Mattila *et al.* (2000).

Determination of cytotoxicity effect:

MTT method was applied on Caco2 and HepG2 by using water propolis and

pollen extract according to the method of Slate *et al.* (1963), Alley *et al.* (1988) and Va de Loosdrecht *et al.* (1994).

Preparation of crackers (as application):

Crackers were prepared as Bose and Shams-Ud-Din (2010) where both extracts (propolis and pollen) were mixed in a dough mixer using the flat beater for 1 minute, scraped down, and continued to mix for 3 min. at high speed. The resulted dough was left to rest for 5 min. Then sheeted to 3mm. Thickness circle pieces cut of dough were formed using the templates with an outer diameter of 5 mm. The crackers were baked at 170 °C for 7 min. After baking, crackers were allowed to cool at room temperature before sensory evaluations.

Table (1): ingredient of crackers samples (g/100gm).

Ingredients	1	2	3	4	5	6	7
Wheat flour	100	100	100	100	100	100	100
Corn oil	10	10	10	10	10	10	10
Salt-	2	2	2	2	2	2	2
Dry milk	1	1	1	1	1	1	1
Baking powder	2	2	2	2	2	2	2
Water extract of propolis	---	0.5	1	1.5	---	---	---
Water extract of pollen	---				0.5	1	1.5

1: Control sample, 2: 0.5% w.propolis ext., 3:1% w.propolis ext., 4: 1.5% w.propolis ext., 5: 0.5% water pollen ext., 6: 1% water pollen ext., 7:1.5% water pollen ext.

Water activity of the crackers:

Water activity was determined using a thermo-hygrometer (HygroPalm HP23-AW, Rotonic AG) at 16±1°C. The measurements were performed in triplicates from powdered samples (Shahidi *et al.*, 2008).

Texture Analysis of crackers sample:

The texture of the crackers was characterized (hardness, adhesiveness and resilience) 24h after baking (Texture Pro CT V1.6BUILD –Brook filed Engineering Labs. Inc).

Sensory evaluation of crackers samples:

The crackers were evaluated for their sensory characteristics after baking by ten panelists from the staff of Bread and Pastry, Research Dept., Food Tech. Res.

Institute, Giza. Each cracker sample was subjected to evaluation with respect to its crispy, odor, taste, color, appearance and overall acceptability (Stone and Sidel, 2004).

Statistical analysis:

Statistical analysis was carried out by SPSS program (Version 19). Data were expressed as means ± SEM and the statistical analysis was performed using one way analysis of variance followed by Duncan's tests (Snedecor and Cochran, 1989).

RESULTS AND DISCUSSION

Total phenols, Total flavonoids and antioxidant activities by DPPH for propolis and pollen and their water extract:

Total phenols, total flavonoids and antioxidant activities by DPPH for propolis and pollen and their water extract are shown in Table (2). The values of the total phenols (16.99 and 19.29 mg/100g) and total flavonoids (38.74 and 43.64 mg/100g) in raw pollen were more than that in raw propolis. Similarly the values of the total phenols and total flavonoids of water extract of pollen were more than that in water extract of propolis. On the other hand, water extract of propolis and pollen had higher antioxidant activity by DPPH than raw propolis and pollen. Moreover,

the water extract of propolis had the highest level of antioxidant activity by DPPH (86.49%). Antioxidant capacity is widely used as a parameter for medicinal bioactive components. In the present study, The antioxidant activity of the extracts was investigated by using DPPH assay method. The results of the DPPH assay emphasize a dose dependent antioxidant activity of the extracts as shown in Table (2). These results are in accordance with Abd El Hady and Hegazi (2002) who found that water propolis extract had higher DPPH than water pollen extract.

Table (2). Total phenols, flavonoids and antioxidant activities by DPPH for propolis, pollen and their water extract (mg/100g).

Sample	T. phenol	T. flavonoids	DPPH
Raw propolis	16.99±0.01	38.74±0.04	58.77±0.77
Raw pollen	19.29±0.03	43.64±0.18	53.02±0.39
Water propolis ext.	7.47±0.31	18.32±0.23	86.49±1.16
Water pollen ext.	8.12±0.00	20.38±0.15	85.98±2.29

Means± slandered Error Means of (triple) three times

Fraction and Identification of phenolic compounds of raw propolis, pollen and their water extract:

HPLC of the raw propolis and pollen and their extract led to the identification of ~16 compounds in each sample. The phenol compounds

concentration in raw propolis and pollen were more than those in their water extracts. Also, raw pollen had the highest level of caffeine, ferulic, ellagic, and oleuropin (36.51, 28.41, and 18.65 and 16.07 mg/100g), respectively as a phenolic compounds (Table 3).

Table (3): Fraction and identification of phenolic compounds of raw propolis, pollen and their water extract (mg/100g).

Compound	Raw		Water extract	
	Propolis	Pollen	Propolis	Pollen
Gallic	1.47	1.50	0.25	0.54
3-OH Tyrosol	0.24	0.25	0.03	0.24
Catechol	8.60	5.02	2.55	1.61
4-Amino benzoic	0.39	0.22	0.05	0.08
Catechin	8.44	9.07	0.75	0.75
Chlorogenic	2.95	3.01	1.35	0.37
P-OH- benzoic	2.17	1.48	0.26	0.31
Benzoic	2.92	1.56	0.28	0.19
Caffeic	3.16	4.51	0.33	1.18
Vanillic	3.17	3.51	0.18	1.19
Caffeine	1.89	36.51	0.36	N.D
Oleuropin	5.01	16.07	0.96	1.43
Ferulic	6.25	28.41	0.19	7.95
Ellagic	14.63	18.56	2.44	5.98
Coumarin	8.84	2.23	N.D	0.55
Pyrogallol	7.92	6.70	N.D	N.D

In Vitro antitumor study on propolis and pollen as honeybee products

Fraction and Identification of Flavonoid compounds of raw propolis, pollen and their water extract:

HPLC of the raw propolis and pollen and their extract led to the identification of ~11 compounds in each sample. The flavonoids compounds

Table (4). Fraction and Identification of Flavonoid compounds of raw propolis, pollen and their water extract (mg/100g).

Compound	Raw		Water extract	
	Propolis	Pollen	Propolis	Pollen
Rutin	4.30	32.88	0.43	2.55
Naringin	25.48	52.34	9.84	50.53
Rosmarinic	0.41	1.89	0.12	0.33
Quercetrin	0.01	3.89	0.32	0.82
Apigenin-7-glucose	8.00	0.01	0.75	2.20
Quercetin	3.38	31.01	N.D	7.25
Naringenin	0.00	9.94	0.63	10.47
Kaemp.3- (2-p-comaroyl) glucose	17.87	122.88	N.D	8.81
Kampferol	2.55	17.50	0.09	1.56
Acacetin 7 neo. rutinoside	12.32	84.70	0.43	7.56
Apigenin	1.07	2.90	0.41	3.89

concentration in raw propolis and pollen were more than those in their water extracts (Table 4). Also, the raw pollen had the highest level of (Kaemp.3- (2-p-comaroyl) glucose and Acacetin 7 neo. rutinoside (122.88 and 84.70 mg/g), respectively as a Flavonoids compounds.

IC50 of water extract of propolis and pollen on caco2 and HepG2:

This study assessed the cytotoxic characteristic of the water propolis and pollen extract against caco2 and HepG2 liver cancer cell line. The results showed a potent anticancer activity of all extracts of propolis and pollen. IC50 value for caco2 was ranged from (123.55-113.61µg/mL) for water propolis and pollen extract, respectively (Table 5).

The cytotoxic activity of water pollen extracts was generally higher than that of water propolis extract (Fig. 1). Many reports have indicated that different types of propolis and pollen extract significantly inhibit cell growth and reduce the differentiation or proliferation of tumor cells (Khalil, 2006; Zliska *et al.*, 2011). Rosmarinic acid as a flavonoid compounds present in propolis and pollen especially raw pollen has antioxidant. Rosmarinic acid helps to prevent cell damage caused by free radicals, thereby reducing the risk

for cancer and atherosclerosis (Hossan *et al.*, 2014). Moreover, Quercetin as a flavonoids compound found in pollen more than in propolis and it has been proven to be a potent component in antioxidant and anticancer against human cancer cell lines, MCF-7, Hep-G2 and NCI-H460 (Son and Anh, 2013).

The main compounds responsible for the anti-tumor activity of propolis include flavonoids, terpenes and caffeic acid phenethyl ester, and this activity could be attributed to synergism between the substances present in the resin (Valente *et al.*, 2011). The possible mechanism of action of propolis against tumor involves apoptosis, cell cycle arrest and interference on metabolic pathways (Watanabe *et al.*, 2011). Also, polyphenols of pollen have been reported to be responsible for their antioxidant activity. Subsequently, reducing the risk of free radicals, genotoxic substance or carcinogenics (Ohta *et al.*, 2007).

Table (5). IC50 of water extract of propolis, pollen on caco2 and HepG2.

Tumor cell	water extract of propolis	water extract of pollen
CaCO2	123.55	113.61
HepG2	352.2	825.25

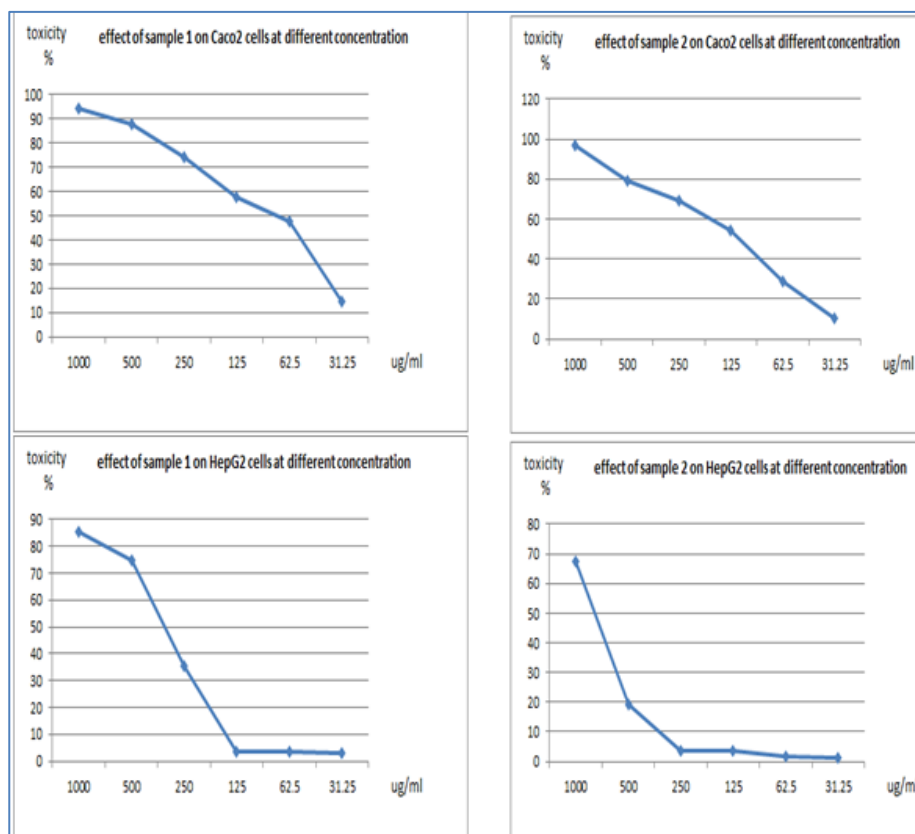


Fig. (1). Effect of water extract of propolis and pollen on caco2cell andHepG2 cell
Sample (1): water propolis extract. Sample (2): water pollen extract

Chemical composition of crackers samples:

The moisture content values of crackers with 0.5%, 1% and 1.5% water propolis and pollen extract were (3.06, 6.24 and 3.57 g) and (4.46, 7.51 and 6.22 g), respectively. Samples of crackers with added propolis were more preferable than

those with propolis due to decreasing in their moisture content (Table 6). The protein content was high in all samples of crackers especially when added 1.5% pollen ext. (38.42mg/100g). Moreover the total carbohydrate decreased in samples when added 1.5% pollen ext. (Table 6).

Table (6). Chemical composition of crackers samples (mg/100g).

Sample	Moisture	Protein	Fat	Ash	T. Carbohydrates	Energy (K cal/1000g)
Addition water propolis extract						
1	5.38±0.72 ^a	22.72±0.20 ^c	4.38±0.14 ^b	3.28±0.06 ^a	64.02±2.98 ^a	397.96 ±.39 ^a
2	3.06±0.02 ^b	29.59±0.32 ^a	2.41±0.08 ^c	3.36±0.14 ^a	61.56±0.18 ^b	386.37±1.39 ^a
3	6.24 ±0.12 ^a	27.39±0.02 ^b	4.54±0.03 ^b	2.80 ±0.06 ^b	59.01±0.9 ^b	386.64±1.21 ^a
4	3.57 ±0.04 ^b	30.38 ±0.39 ^a	5.53 ±0.18 ^a	3.46±0.09 ^a	57.06 ±0.56 ^b	399.53 ±2.95 ^a
Addition water pollen extract						
1	5.38±0.72 ^{bc}	22.72±0.20 ^d	4.38±0.14 ^c	3.28±0.06 ^b	64.02±2.98 ^a	397.96 ±.39 ^a
5	4.64 ±0.05 ^c	35.02 ±0.04 ^c	7.64 ±0.1 ^a	3.49 ±0.0 ^a	49.02±0.0 ^b	404.92 ±.23 ^a
6	7.51±0.02 ^a	37.23±0.16 ^b	6.21±0.13 ^b	2.99±0.05 ^c	46.06±0.01 ^b	389.05 ±1.23 ^a
7	6.22±0.00 ^b	38.42 ±1.9 ^a	7.83±0.10 ^a	3.39 ±0.04 ^c	44.14±0.56 ^b	400.71 ±.49 ^a

1: Control sample, 2: 0.5% w. propolis ext., 3: 1% w. propolis ext., 4: 1.5% w. propolis ext., 5: 0.5% w. pollen ext., 6: 1% w. pollen ext., 7: 1.5% w. pollen ext.

a,b,c: values are mean±SE (n=3). The mean values within a column indicate significant differences (p<0.05). LSD is the least significant difference.

Total phenol, Total Flavonoids and Anti-oxidant activities of crackers samples:

The anti-oxidant activity increased in crackers samples fortified with 0.5% to 1.5% of water propolis and pollen extract (Table 7). Also, the values of the total phenolic and flavonoids content of crackers containing 0.5% to 1.5% of water propolis and pollen extract increased with increasing the percent of propolis and pollen extract comparative to the control sample. The value of 2,2-Diphenyl-1-

picrylhydrazyl (DPPH) isoflavone is high in crackers sample which fortified with 1, 5% water propolis extract (Table 7). This result is due to the presence of high antioxidant activity in propolis and pollen according to LeBlanc *et al.* (2009) and de Florio *et al.* (2017). Also, Agati *et al.* (2012) and Nisar (2022) reported that bee pollen contains a high concentration of phenolic components such as cinnamic acid derivatives, flavonoids, flavones, isoflavones, anthocyanins, and flavonols.

Table (7): Total phenol, Total Flavonoids and Anti-oxidant activities of Crackers samples (mg/100g).

Sample	T.Phenol	T.Flavonoids	DPPH
Addition extract of propolis			
1	11.86±0.49 ^c	33.58±0.33 ^c	26.47±1.10 ^b
2	11.45±0.06 ^c	34.13±1.32 ^c	36.91±0.81 ^b
3	13.14±0.52 ^b	35.20±1.27 ^c	38.90±0.19 ^b
4	13.87±0.26 ^b	34.42±0.19 ^c	39.85±5.53 ^a
Addition of water extract of pollen			
1	11.86±0.49 ^a	33.58±0.33 ^c	26.47±1.10 ^a
5	14.24±0.50 ^b	30.86±0.30 ^d	33.59±0.85 ^b
6	14.76±0.06 ^b	35.03±0.46 ^b	38.52±1.08 ^a
7	14.88±0.10 ^b	37.27±0.32 ^a	41.16 ±0.48 ^a

1: Control sample, 2: 0.5% w. propolis ext., 3:1% w. propolis ext., 4: 1.50% w. propolis ext., 5: 0.5% w.pollen ext., 6: 1% w. pollen ext., 7:1.50% w.pollen ext.

a,b,c: values are mean±SE (n=3). The mean values within a column indicate significant differences (p≤0.05). LSD is the least significant difference.

Sensory evaluation of crackers samples:

The most widely used scale for measuring food acceptability through senses is the 5-point hedonic scale. This scale was used for evaluating the sensory properties of the crackers. Seven variations of the crackers were developed by the incorporation of water propolis and pollen extract. The odor was improved with the addition of water propolis and pollen extract, especially pollen ext. (Table 8). Taste had high value when added 1.5% water pollen extract. The increase in percent of addition of water propolis and pollen extract had a positive effect on

crispy of crackers where the highest score was recorded with samples with 1.5% water pollen extract. Color had lower value when added water propolis extract, while it had a high value with addition of water pollen extract (Table 8). Moreover, the general appearance of crackers was acceptable after addition of 1.5% from both extract. Adding 1.5% of water pollen extract improved all sensory evaluation parameters more than addition of water propolis extract. This result is in accordance with that of AL-Kahtani (2017) who used bee pollen and produce biscuit.

Table (8). Sensory evaluation of crackers samples .

No	Odor	Taste	Crispy	Color	general appearance
Addition water propolis extract					
1	17.60±0.52 ^a	17.50±0.40 ^a	17.80±0.48 ^a	18.30±0.47 ^{ab}	18.10±0.43 ^a
2	18.00±0.36 ^a	18.00±0.39 ^a	17.70±0.51 ^a	17.50±0.37 ^b	17.50±0.42 ^a
3	18.30 ±0.26 ^a	18.30 ±0.30 ^a	18.20 ±0.41 ^a	18.90 ±0.27 ^a	18.80 ±0.29 ^a
4	18.10 ±0.27 ^a	18.00 ±0.49 ^a	18.70±0.21 ^a	17.70±0.50 ^b	17.90 ±0.56 ^a
Addition water pollen extract					
1	17.60±0.52 ^b	17.50±0.40 ^b	17.80±0.48 ^b	18.30±0.47 ^{ab}	18.10±0.43 ^b
5	18.50 ±0.34 ^{ab}	18.30±0.44 ^{ab}	18.70 ±0.33 ^{ab}	51 ^b . 17.70±	18.30±0.47 ^{ab}
6	18.30±0.26 ^{ab}	18.20±0.20 ^{ab}	18.70±0.30 ^{ab}	18.60±0.22 ^{ab}	18.70±0.26 ^{ab}
7	18.80 ±0.24 ^a	19.10±0.23 ^a	19.00±0.29 ^a	19.40±0.16 ^a	19.30±0.21 ^a

1: Control sample, 2: 0.5% w.propolis ext., 3:1% w.propolis ext., 4: 1.5% w.propolis ext., 5: 0.5% w. pollen ext., 6: 1% w.pollen ext., 7:1.5% w.pollen ext.

a,b,c: values are mean±SE(n=3). The mean values within a column indicate significant differences (p≤0.05).LSD is the least significant difference.

Texture analysis and water activity of crackers samples:

The impact of 0.5%, 1% and 1.5% of adding water propolis extract and 0.5%, 1% and 1.5% water pollen extract had been investigated on the hardness and time required for break cracker samples. The results indicated that hardness was decreased with increasing the percent of adding water propolis extract compared to the control sample. The higher value of adhesiveness was observed in samples with high percent of both propolis and pollen extract. There was no change in resilience of all samples compared to control sample except those with addition 0.5% water propolis extract.

A significant decrease in water activity was observed in the cracker enriched with water propolis extract (1-1.5%) compared to the control sample. Similar results were observed in studies conducted by Min *et al.* (2016) and Zielinska *et al.* (2020). Likewise, there was a consistent reduction in water activity with the addition of water propolis extract. While the value of water activity with addition pollen extract decreased from (0.42) in control sample to (0.043, 0.047, 0.045) in crackers sample with addition of 0.5%, 1% and 1.5% water pollen extract, respectively

Table (9). Texture analysis and water activity of crackers samples.

NO	Hardness Cycle1N	Texture analysis			Water activity
		Adhesiveness MJ	Resilience	Fractureability N	
Addition water propolis extract					
1	64.46	0.00	0.01	57.71	0.42
2	85.96	0.30	0.22	32.03	0.60
3	35.87	0.00	0.00	14.09	0.27
4	28.36	0.80	0.00	17.14	0.30
Addition of water pollen extract					
1	64.46	0.00	0.01	57.71	0.42
5	63.71	0.10	0.01	27.41	0.043
6	68.97	0.00	0.00	46.86	0.047
7	75.42	0.80	0.01	14.86	0.045

1: Control sample, 2: 0.5% water extract of propolis, 3:1% water propolis ext., 4: 1.5% w.propolis ext., 5: 0.5% w. pollen ext., 6: 1% w. pollen ext., 7:1.5% w. pollen ext.

Conclusion:

It was concluded from this study that addition of propolis and pollen as powder to special food gave a better results compared to their water extract in treatment of tumor cell in in vitro. Also, water extract of propolis and pollen enhanced chemical composition of crackers with respect to protein, ash, total carbohydrate and fat. Crackers had high value from antioxidant (total phenol, flavonoid, DPPH) and enhanced sensory value of flavor- taste- textalue - color - general appearance when add high percent 1.5% from both extracts.

REFERENCES

- A.O.A.C. (2000). Official Method of Analysis of the Association of the Analytical Chemsits. 17ed Published by the Assoication of Official Analytical Chemists. PO Box 540. Benjamin Franklin Station Washington DC. 20044.
- Abd El Hady, F.K. and Hegazi A.G. (2002). Egyptian propolis: 2. Chemical composition, antiviral and antimicrobial activities of east Nile delta propolis. *Zeit.Naturforsch*, 57: 386-394
- Agati, G.; Azzarello, E.; Pollastri, S. and Tattini, M. (2012). Flavonoids as antioxidants in plants: location and functional significance. *Plant Sci.*, 196:67-76
- AL-Kahtani, S.N. (2017). Fatty Acids and B Vitamins Contents in Honey Bee Collected pollen in relation to botanical origin. *Scientific J. King Faisal Univ. (Basic Appl. Sci.)*, 18(2): 41-48.
- Alley, M.C.; Scudiero, D.A.; Monks, A.; Hursey, M.L.; Czerwinski, M.J.; Fine, D.L. and Boyd, M.R. (1988). Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. *Cancer Res.*, 48 (3):589-601.
- Anjuma, S.I.; Ullah, A.; Khan, K.A.; Attaullah, M.; Khan, H.; Ali, H.; Bashir, M.A.; Tahir, M.; Ansari, M.J.; Ghramh, H.A.; Adgaba^N and Dash, C.K (2019). Composition and functional properties of propolis (bee glue) : A review. *Saudi J. Biol. Sci.*, 26:1695–1703
- Batista, C; Barros, L; Carvalho, AM and Ferrira, ICFR.(2011). Nutritional and nutraceutical potential of rape (*Brassica napus* L. *vornapus*) and "tronchuda" cabbage (*Brassica oleraceae* L. var. *Costata*) inflorescences. *Food Chemical Toxicol.*, 49:1208-1214.
- Bose, D. and Shams-Ud-Din, M, (2010). The effect of chickpea (*Cicera rietinim*) husk -on the properties of cracker biscuits. *J. Bangladesh Agri. Univ.* 8 (1) :147-152.
- de Florio A.J.; Reis A.S.D.; Heldt, L.F.S. and Pereira, D. (2017). Lyophilized bee pollen extract: a natural antioxidant source to prevent lipid oxidation in refrigerated sausages, 76:299–305.
- de Lima, G.G.; de Souza, R.O.; Bozzi, A.D.; Poplawska, M.A.; Devine, D.M. and Nugent, M.J.D. (2016). Extraction method plays critical role in antibacterial activity of propolis-loaded hydrogels. *J. Pharm. Sci.*, 105:1248–1257
- Goupy, P.; Hugues, M.; Biovin, P. and Amiot, M.J. (1999). Composition and activity of borley (*Hordeum vulgare*) and malt extracts and of isolated phenolic compounds *J. Sci. Food Agric.*, 79:1625-1634.
- Han, J.; Janz, J.A.M. and Gerlat, M. (2010). Food Development of gluten-free cracker snacks using pulse flours and fractions. *Res. Int.*, 43: 627- 633.
- Hanato, T; Magawa, H.; Yasuhara, T. and Okuda, T. (1988). Two new flavonoids and other constituents in licorice root: their relative a

- stringency and radical scavenging effects. *J. Chem. Pharm. Bull.*, 36: 2090-2097.
- Hossan, S.; Rahman, S.; Bashar, A.B.M.A.; Jahan, R.; Al-Nahain, A. and Rahmatullah, M. (2014). Rosmarinic acid: a review of its anticancer action. *World J. Pharm. Pharmaceutical Sci.*, 3(9):57–70.
- Huang, S.; Zhang, C.P.; Wang, K.; Li, G.Q.; and Hu, F.L. (2014) Recent advances in the chemical composition of propolis. *Molecules*, 19:19610–19632
- Ivan L.L.; Okhee, Y.L.; Yong, L.; Katherine, H. and Cornelia, L. (2021). Optimisation of bee pollen extraction to maximise extractable antioxidant constituents. *MDPI*, 10(7):1113.
- Khalil, M.L. (2006). Biological activity of bee propolis in health and disease. *Asian Pacific J. Cancer Prevention*, 7(1):22–31
- Kim, A.M.; C.R. Barry; R. Burke; K. Hussey; S. McCarthy and E. Gallagher (2017). Effect of pulse flours on the physiochemical characteristics and sensory acceptance of baked crackers. *School of Food Science and Environmental Health*, 03-29
- LeBlanc B. W., Davis O. K., Boue S., DeLucca A., Deeby T, (2009). Antioxidant activity of Sonoran desert bee pollen. 115 (4) :1299–1305. doi: 10.1016/j.foodchem.2009.01.055.
- Mattila, P.; Astala, J. and Kumpulainen, J. (2000). Determination of flavonoids in plant material by HPLC with diode-array and electro-array detections. *J. Agric. Food. Chem.*, 48: 5934-5941.
- Min, K.T.; Kang, M.S.; Kim, M.J.; Lee, S.H.; Han, J.S. and Kim, A.J. (2016) Manufacture and Quality Evaluation of Cookies prepared with Mealworm (*Tenebrio molitor*) Powder. *Korean J. Food Nutr.* 29:12–18.
- Munsted, K. and Bogdanov, S. (2009). Bee products and their potential use in modern medicine. *J. Api Prod. Api Med. Sci.*, 1:57–63.
- Nisar, A. (2022). Medicinal Plants and Phenolic Compounds. *Phenolic Compounds: Chemistry, Synthesis, Diversity, Non-Conventional Industrial, Pharmaceutical and Therapeutic Applications*, 131.
- Ohta, S.; Fujimaki, T.; Uy, M.M.; Yanai, M.; Yukiyoishi, A. and Hirata, T. (2007). Antioxidant hydroxyl-cinnamic acid derivatives isolated from Brazilian bee pollen. *Nat. Prod. Res.*, 21(8):726–32
- Pascoal, A.; Rodrigues, S.; Teixeira, A.; Feás, X. and Estevinho, L.M. (2014). Biological activities of commercial bee pollens: antimicrobial, antimutagenic, antioxidant and anti-inflammatory. *Food Chem. Toxicol.*, 63:233–239.
- Shahidi, F; Sedaghat, N; Farhoush, R, and Monsavi-Nik, H. (2008). Shelf life determination of saffron stigma: water activity and temperature studies. *World Appl. Sci. J.*, 5(2):132-136.
- Slater, T.F.; Sawyer, B. and Sträuli, U. (1963). Studies on succinate-tetrazolium reductase systems: III. Points of coupling of four different tetrazolium salts III. Points of coupling of four different tetrazolium salts. *Biochim. Biophys. Acta*, 77:383-393.
- Snedecor, G.W. and Cochran, W.G. (1989). *Statistical Methods*. The Lowe State University Press. Ames, Lowe.
- Son, H.L. and Anh, N.P. (2013). Phytochemical composition, in vitro antioxidant and anticancer activities of quercetin from methanol extract of *Asparagus cochinchinensis* (LOUR.) Merr. *Tuber. J. Med. Plants Res.*, 7(46):3360–3366.
- Stone, H. and Sidel, J. (2004). *Sensor evolution practices*, 3rd Edition, Academic Press, London.

- Sudha, M.L.; Vetrinani, R. and Leelawath, I.K. (2007). Influence of fiber from different cereals on the rheological characteristics of wheat flour dough and on biscuit quality. *Food Chemistry*, 100 (4):1365-1370.
- Szczesna T. (2006). Protein content and amino acid composition of bee-collected pollen from selected botanical origins. *J. Apic. Sci.*, 50:81-90
- Valencia, V.N.; Granados, P.E.; Agama, A.E.; Tovar, J.; Ruales, J. and Bello, P.L.A. (2006). Fiber concentrate from mango fruit: Characterization, associated antioxidant capacity and application as a bakery product ingredient. *LebensmittelWissenschaft und-Technologie*, 40(4):722-729.
- Valente, M.J.; Baltazar, A.F.; Henrique, R.; Estevinho, L. and Carvalho, M. (2011). Biological activities of Portuguese propolis: Protection against free radical-induced erythrocyte damage and inhibition of human renal cancer cell growth in vitro. *Food Chem. Toxicol.*, 49: 86-92.
- Van de Loosdrecht, A.A.; Beelen, R.H.J.; Ossenkoppele, G.; Broekhoven, M.G.; Langenhuijsen, M.M.A.C. (1994). A tetrazolium-based colorimetric MTT assay to quantitate human monocyte mediated cytotoxicity against leukemic cells from cell lines and patients with acute myeloid leukemia. *J. Immunol. Methods*, 174(1-2): 311-32.
- Watanabe, M.A.E.; Amarante, M.K.; Conti, B.J and Sforcin, J.M. (2011). Cytotoxic constituents of propolis inducing anticancer effects: a review. *J. Pharm. Pharmacol.*, 63: 1378-1386
- Zielinska, E. and Pankiewicz, U. (2020). Nutritional, Physiochemical, and antioxidative characteristics of shortcake biscuits enriched with TenebrioMolitor Flour. *molecules MDPI Journals.*, 25(23) :pages 4-13.
- Zliszka, E.; Czuba, Z. P.; Bronikowska, J.; Mertas, A.; Paradysz, A and Krol, W. (2011). Ethanolic extract of propolis augments TRAIL-induced apoptotic death in prostate cancer cells. Hindawi publishing Corporation.

دراسة مضادة للأورام في المختبر على البروبوليس وحبوب اللقاح كمنتجات لنحل العسل

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المستخلص

تهدف الدراسة الحالية في المختبر إلى التحقق من قدرة البروبوليس وحبوب اللقاح كمنتجات عسل النحل في علاج الورم. وكذلك استخدامه ومستخلصه المائي كغذاء وظيفي له خصائص دوائية ومركبات مضادة للأكسدة. تمت دراسة هذا البحث على الخلايا السرطانية (خلية Caco2 وخلية hepG2) في دراسة مضادة للأورام في المختبر. تم تقدير التركيب الكيميائي والفينولات الكلية والفلافونويدات الكلية ونشاط مضادات الأكسدة DPPH. تم تحديد تجزئة وتحديد المركبات الفينولية ومركبات الفلافونويد بواسطة HPLC. وكذلك استخدام المستخلصات المائية من البروبوليس وحبوب اللقاح في منتج المقرمشات. أوضحت النتائج ان البروليس الخام وحبوب اللقاح كانت أعلى فاعلية من المستخلصات المائية لمجموع الفينولات والفلافونويدات الكلية. في حين كان المستخلص المائي للبروبوليس أعلى نشاطاً مضاداً للأكسدة بواسطة DPPH مقارنة بالعينات الخام. وعلاوة على ذلك، كان محتوى البروبوليس وحبوب اللقاح الخام أكثر من المركبات الفينولية والفلافونويدية مقارنة بمستخلصهما المائي بواسطة HPLC. كما أن حبوب اللقاح الخام تحتوي على أعلى مستوى من الكافيين، الأوليوربين، الفيروليك والإلاجيك (٣٦.٥١، ١٦.٠٧، ٢٨.٤١، ١٨.٦٥ ملجم/١٠٠ جم) كمركبات فينولية. أوضح فحص مضاد للورم ان قيمة IC50 للمستخلص المائي للبروبوليس (١٢٣.٥٥) أكثر من المستخلص المائي لحبوب اللقاح (IC50=١١٣.٦١) لسرطان القولون (caco2). في حين أن المستخلص المائي لحبوب اللقاح (IC50=٨٢٥.٢٥) أكثر من المستخلص المائي للبروبوليس (IC50=٣٥٢.٢) لسرطان الكبد (HepG2). كانت عينات البسكويت التي تحتوي على نسبة عالية (١، ٥٪) من مستخلص الماء من البروبوليس أو حبوب اللقاح تتمتع بأعلى درجات الطعم والنكهة والملمس والمظهر العام. ويستنتج من الدراسة انه يمكن استخدام دنج النحل كمضاد لسرطان القولون وحبوب لقاح النحل كمضاد لسرطان الكبد. وكذلك يمكن استخدام البروبوليس وحبوب اللقاح في الأغذية الوظيفية.